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Anti-parasitic activity of garlic (*Allium sativum*) and onion (*Allium cepa*) juice against crustacean parasite, *Lernantropus kroyeri*, found on European sea bass (*Dicentrarchus labrax*)

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ABSTRACT

The copepod parasite *Lernantropus kroyeri* impairs the productivity of European sea bass (*Dicentrarchus labrax*) culture by leading to a direct infection or as a vector for other disease agents. In current conditions, the parasitic infections in mariculture are usually treated with the chemicals, mainly with the pesticides. However, therapeutic chemical use has potential risks on the overall aquatic environment. Thus, the aquaculture sector needs to find alternative substances to the treatment or prophylaxis of the diseases instead of chemicals. In the present study, *in vitro* exposure tests were performed to elucidate the effects of garlic (*Allium sativum*) and onion (*Allium cepa*) on adult *L. kroyeri*. Female individuals of *L. kroyeri* were exposed to different concentrations of garlic or onion juice (20, 40, 60, 80 and 100% of the plant juice) for a specified time. Garlic juice exposure at a ratio of 100% resulted in instantaneous death of all individuals in five minutes. Although the killing time varied by the concentration, garlic juice killed the *L. kroyeri* females within 30 min even at the lowest concentration. Onion juice had lower killing effect at low concentrations, however, higher concentrations of onion juice killed the female parasites within 60 min. Results revealed that garlic and onion juices had killing effect on *L. kroyeri* females in a time and concentration-dependent manner. It can be concluded that against parasitic copepods alternative solutions can be developed in aquaculture, using these plant juices with emphasising necessity of further *in vivo* tolerance tests in fish.

HIGHLIGHTS

- Garlic and onion juices had anti-parasitic effect *in vitro* on the crustacean parasite, *Lernantropus kroyeri* found on the gills of European sea bass (*Dicentrarchus labrax*).
- After exposure of *L. kroyeri* to garlic and onion juices, the mortality was time and concentration-dependent.
- The killing activity of garlic juice for *L. kroyeri* was stronger than that of onion juice.

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Introduction

Copepod parasites as all other parasites have serious negative impacts on aquaculture production with considerable economic loss. *Lernantropus* is a frequently observed parasitic copepod genus in cultured European sea bass (*Dicentrarchus labrax*) in the Mediterranean region. *Lernanthropus kroyeri* (Copepoda, Siphonostomatoida: Lernanthropidae) is a gill copepod and *L. kroyeri* invasions have previously been reported in cage-cultured European sea bass in various locations (Antonelli et al. 2012; ER and

KAYIS 2015). The morphological characteristics of *L. kroyeri* and the pathological effects on the European sea bass have also been well documented (Özel et al. 2004; Toksen 2007). Copepod parasites destroy the tissue, feed on the mucus, skin and blood of the fish, causing osmoregulation stress, impaired feed conversion and increase in secondary infections associated with weakening of the fish (Misganaw and Getu 2016).

The intensification of aquaculture practices creates appropriate conditions for the over-growth of parasites in the aquatic environment. Parasite elimination

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Table 1. Dosage formulation for the plant juice (garlic or onion) concentrations.

Trial group	Volume of garlic/onion juice, μL	Volume of seawater, μL	Final percentage of garlic or onion juice, %
I	250	0	100
II	200	50	80
III	150	100	60
IV	100	150	40
V	50	200	20
Control	0	250	0

strategies in intensified aquaculture have been neglected over the years. Most of the research has focussed on how to reduce financial losses caused by parasitic infections of farmed fish and the practical solutions are mostly driven by short-term chemical treatment targeting the elimination of the parasites directly or reducing transmission.

Several pesticides were the most common applied agents to treat the parasitic crustaceans in aquaculture. The control of the parasitic crustaceans such as *Caligus rogercresseyi* in salmon industry relies mainly on chemical treatments, with reported resistance to deltamethrin and emamectin benzoate (Agusti et al. 2016). In sea-cage cultured European sea bass emamectin benzoate has been used in the control of *L. kroyeri* (Toksen et al. 2006). Today, emamectin benzoate is still being used in marine aquaculture of sea bass against the crustacean parasites. However, the treatment of the disease agents using natural substances is required to be compatible with sustainable aquaculture elements. In approaching to solve the disease problems in aquaculture one of the reasonable solutions is the using of plant extracts, including those from garlic and onion, as natural treatments or prophylaxis. The studies on the effects of plant-derived substances on fish parasites have been widely reviewed in the literature (Picón-Camacho et al. 2012; Erguig et al. 2015; Wunderlich et al. 2017; Tavares-Dias 2018). Anti-parasitic activities of garlic and onion specifying the anti-protozoan, anti-myxozoan and anthelmintic activities were studied in different parasites and fish (Martins et al. 2002; Buchmann et al. 2003; Picón-Camacho et al. 2012; Fridman et al. 2014; Wunderlich et al. 2017; Hyun Kim et al. 2019). To our knowledge, the anti-crustacean parasite activity of garlic and onion was not studied in aquaculture. Therefore, we carried out preliminary *in vitro* tests to evaluate the anti-crustacean parasitic effect of garlic or onion juices on *L. kroyeri* female individuals.

Material and methods

European sea bass was obtained from a commercial marine aquaculture company located in Kazikli Bay (Mugla, Turkey). The females with egg sac of the

crustacean parasite *L. kroyeri* were carefully removed from sea bass gills by using forceps, as soon as fish were harvested and died. A garlic press was used to get liquid from garlic and onion bulbs which were purchased from the local market. Five concentrations of the plant juice (fresh garlic or onion) and control (seawater) were tested. Groups of 10 individuals were placed in each of the 96-well plates containing the amount of filtered seawater needed to dilute the working solution. After a 1–3 min acclimation, garlic or onion juices at different concentrations were added to each well to achieve the final plant juice ratio (Table 1), in four replicates. For controls, 250 μL filtered seawater was used. Parasites were observed for their motility over 60 min, under the microscope. At the time of no movement to touch with a needle, the parasites were considered as dead. Adverse effects were considered as reduced movement and tremors with contraction.

For the garlic-exposed groups, the parasites were examined at 4 time periods: (1) 5 min immediately after adding the garlic juice to the well (immediate), (2) 10 min, (3) 15 min and (4) 30 min (final) after adding the garlic juice to the well. For the onion-exposed groups the examinations were done at 5 time periods: 5, 10, 15, 30 and 60 min after adding the onion juice to the well.

Statistical analysis to detect the differences in cumulative mortality for the concentrations and exposure time as previously defined was conducted using one-way ANOVA. Differences were considered statistically significant at $p < .05$.

This study was conducted *in vitro* and complies with the ethics of experimentation.

Results and discussion

The current study aimed to research the effects of the use of garlic (*Allium sativum*) and onion (*Allium cepa*) juices against the crustacean parasite *L. kroyeri* female individuals *in vitro*, in order to provide a base for possible alternatives and natural treatments of crustacean parasites. In the present investigation, we report that both garlic and onion juices exerted a strong anti-parasitic crustacean activity.

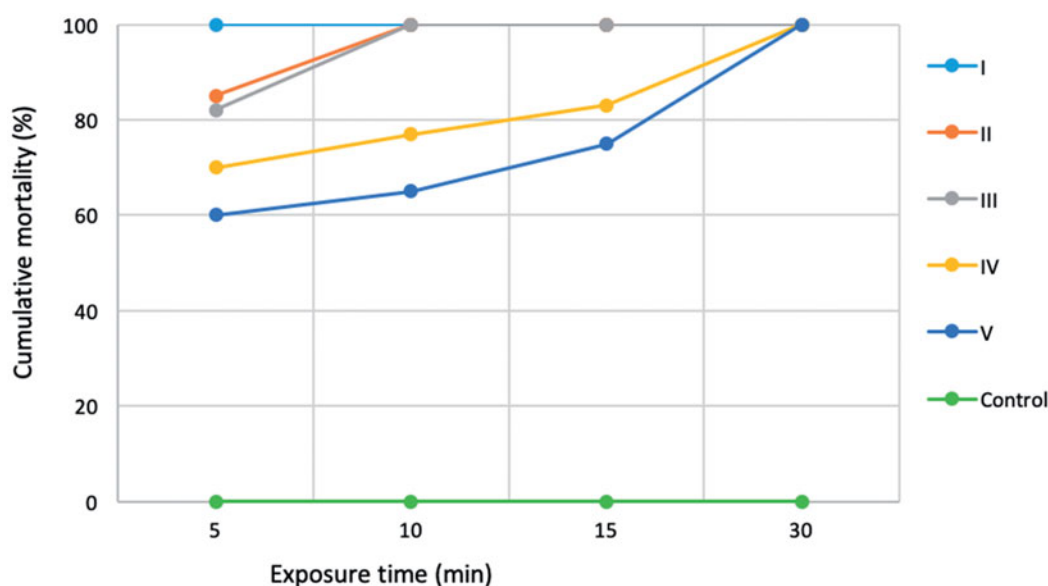


Figure 1. Effect of garlic juice on the mortality of female *Lernantropus kroyeri*. Garlic juice was used at a ratio of 100 (I), 80 (II), 60 (III), 40 (IV), 20 (V) and control (100% seawater).

In our tests, cumulative mortality of *L. kroyeri* females varied by the garlic or onion juice concentrations and exposure time (F (critical value 2.31)=275.67 for garlic experiment and F (critical value 2.29)=9.73 for onion experiment, $p < .05$). In garlic experiments, by 30 min after exposure to garlic juice, the cumulative parasite mortality reached 100% in all groups. The quickest parasite mortality was observed at a ratio of 100% in Group I in 5 min in garlic experiments (Figure 1). In the garlic-exposed groups, the maximum elapsed time to the death for the minimum concentration (20% garlic) was recorded at 30 min. The anti-parasitic effect of garlic in different fish species was studied. The garlic effect was evaluated by Hyun Kim et al. (2019) in guppy infected with the ciliate parasite *Cryptocaryon irritans*. Garlic had an anti-parasitic potential to *C. irritans* *in vitro*, however, *in vivo* experiments with applying garlic orally or by immersion route to fish revealed that the effect was not similar to the results obtained in *in vitro* experiments. In another study, the effect of garlic in the feed on *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) was the reduction of parasites from the gills of pacu (Martins et al. 2002). On the other hand, water-ethanol extracts of garlic and onion applied as immersion were ineffective to the fish parasite *Neobenedenia* sp. (Trasviña-Moreno et al. 2019). In another experiment, the killing capacity of garlic extract on *Ichthyophthirius multifiliis* was dependent on the concentration of garlic extract (Buchmann et al. 2003). In a study performed on gilthead sea bream (*Sparus aurata*), the efficacy tests of garlic against to

the monogenean *Sparicotyle chrysofhrui* found on the gills revealed that garlic was ineffective in killing these parasites (Villar-Torres et al. 2014).

In our tests, parasites treated with onion juice showed a time- and dose-dependent response as observed in the garlic-exposed groups (Figure 2). In onion exposed groups the time elapsed to the death was longer than in the garlic exposed groups, reaching 60 min even in the groups treated with higher levels of onion juice concentrations (60, 80 and 100%). In the onion-exposed groups at the ratio of 40 and 20%, the mortality rate was 50% after 60 min. The studies on onion (*A. cepa*) effects on fish parasites are relatively scarce when compared to those regarding the garlic effects on fish parasites. A destroying impact of onion on myxozoan parasite found in tilapia was reported (El-Mansy and Mohamed 2003).

The phytochemical analysis of the garlic and onion revealed the presence of biologically active compounds, thiosulfinates, phenolic and steroidal substances, showing a wide range of activities such as antibacterial, antiviral, antifungal and antiprotozoal effects (Harris et al. 2001; Lanzotti 2006; Mikaili et al. 2013). There is no any previous study on the action mechanism of garlic or onion on crustacean parasites of fish. However, the quick death of *L. kroyeri* individuals after garlic or onion juice exposure may be linked to the breakdown of nervous system of the parasite. Hence, Chaubey (2017) reported that essential oils of *A. sativum* have neurotoxic effect on insects, causing rapid paralysis and killing effects. It has been ascertained that garlic with more sulphur compounds than

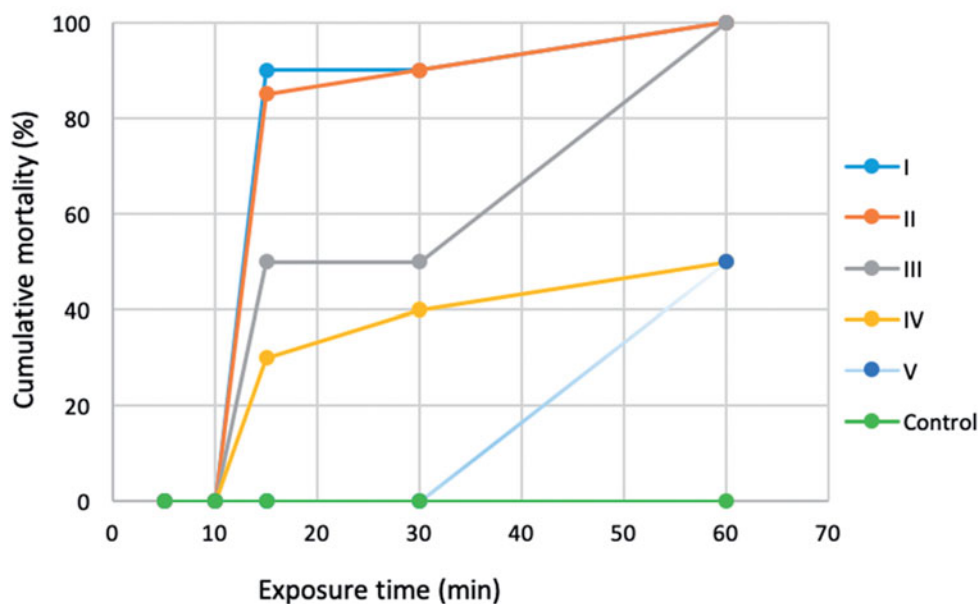


Figure 2. Effect of onion juice on the mortality of female *Lernantropus kroyeri*. Garlic juice was used at a ratio of 100 (I), 80 (II), 60 (III), 40 (IV), 20 (V) and control (100% sea water).

onion exhibits stronger activity against the pathogens (Krstin et al. 2018). Compatibly, in our study, we detected the more strong effect of garlic than onion regarding the killing time of *L. kroyeri*. Furthermore, *L. kroyeri* individuals immediately showed abnormal behaviour, such as strong contraction as soon as they were in contact with garlic or onion juice.

Lernantropus kroyeri is a copepod parasite, infecting mainly cage-cultured European sea bass (*D. labrax*) and leading to economic losses in the marine aquaculture industry. Considerable dimensions of parasite problems have been defined (Agusti et al. 2016). Despite the crucial roles of the parasite to sustain the aquaculture, the parasite elimination has been neglected. There is no a well- developed action plan that had been taken in order to prevent parasitic diseases, thus the existing methods for the prevention of copepod parasites attacks are mainly based on the pesticide application. In general, *L. kroyeri* in the European sea bass aquaculture industry is controlled by the chemical treatments with broad-spectrum pesticides. Natural treatments of fish diseases with various plant extracts have received much more attention in recent years, whereas plant extracts against the crustacean parasites infecting fish have not been studied. Due to challenges in chemicals used for disease treatment in aquaculture, it is considered the fact that the plants can be utilised in the treatment of various fish disease agents, as an alternative to chemicals hazardous for human and environment (Sahandi et al. 2012). In the present study, garlic and onion as plant extracts were proven to their anti-crustacean parasitic capacity

in vitro. The efficacy of garlic juice to *L. kroyeri* was higher. This may be related to the amount and the specific profile of bioactive metabolites present in the garlic. This study describes the efficacy of garlic and onion juices against the crustacean parasite *L. kroyeri* firstly, thus making it possible to develop the new prophylactic agents against parasitic crustaceans in aquaculture.

Conclusions

Time and dose-dependent killing effects of garlic and onion juice on *L. kroyeri* collected from the cage-cultured European sea bass (*D. labrax*) were indicated by this study results. Both plants as applied in fresh juice form showed potential for alternative treatment to manage copepod parasitic disease in aquaculture. However, the identification of active compounds available in garlic and onion bulbs that are specifically involved in killing the crustacean parasites should be done as a next step. This is the first study reporting the killing effect of garlic or onion juices on the copepod parasite *L. kroyeri* obtained from European sea bass. Further investigation is necessary to test the tolerance of fish to these plant extracts and to reveal the active metabolites of garlic and onion that have killing activity against the crustacean parasites.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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